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Vitamin E ameliorated the toxicological effects of crude oil on hematological properties of the growing pig without selenium

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ABSTRACT

Ameliorative effects of vitamin E or in combination with selenium on hematological characteristics of growing pigs fed crude oil-contaminated diets were studied. 36 landrace pigs were randomly allotted to their pens and pre-conditioned for one week. Four dietary crude oil diets: Diet 1, diet 2, diet 3 and diet 4 contained 0 gram, 5 grams, 10 grams and 15 grams of crude oil/kg of diet were fed for 4 weeks to 9 pigs/dietary treatment group tagged the toxicological phase. After this phase, the amelioration phase with vitamin E alone or in combination with selenium with 3 replications for each treatment group involving the transition of the animals from the toxicological phase to the amelioration phase for duration of 4 weeks after which blood samples were collected from all pigs for hematological analyses. Results showed that vitamin E alone ameliorated the toxic effects of crude oil ingestion on the hematological parameters of the growing pig as there were no significant ($P > 0.05$) differences on hematological data of the positive control groups compared with the negative control group for all treatment groups; furthermore, the combination of vitamin E and selenium had no additional benefits on the hematological parameters of the pigs as there were no significant ($P > 0.05$) differences between the vitamin E and vitamin E + selenium groups, respectively. It was thus concluded that vitamin E alone ameliorated the toxic effects of crude oil ingestion on all the growing pig hematological parameters studied in all the treatment groups.

Keywords: Crude oil, Amelioration, Hematological parameters and the Pig.

1. INTRODUCTION

It has been established that one of the areas crude oil ingestions demonstrates some of its toxicological effects is on the blood chemistry of the animal, such as the pig by altering its normal morphology leading to anemia and leukemia (Okejim et al., 2020). To this extent therefore, it is a well-known fact that one of the major means of determining an animal physiological, nutritional status as well as its health condition is via its blood parameters. Thus, morphological

changes in the normal constituents of the blood are usually used as one of the major determinants of the health status of the animal. It is in this direction that the studies of Achuba, (2018) demonstrated that hematology and serum chemical assay aid in determining the physiological disposition of the animal in its environment.

The literature is awash with the efficacy of vitamin E in protecting animals from toxic environmental factors, such as the one caused by crude oil (Val and Almeidi-Val, 1999) due to crude oil capability in generating free radicals in the tissues of the animal (Achuba, 2018) that causes damages to critical cellular macromolecules, including DNA, lipids and proteins. Further studies, such as those of Aslam et al., (2010) showed that the efficacy of the protective effects of vitamin E was increased when it was combined with selenium (Se). This was actually related to the synergistic effect that exists between vitamin E and selenium stimulating the glutathione defense system in protecting the animal. Hitherto, there is no study that has tested the hypothesis of the combined protective effect of vitamin E and selenium on the hematological properties in pigs fed crude oil-contaminated diets. Therefore, the objectives of this study are to test the hypothesis of the efficacy of the protective effect of vitamin E alone or vitamin E in combination with selenium in ameliorating the toxicological effects of crude oil on the hematological characteristics in growing pigs fed crude oil-contaminated diets.

2. MATERIALS AND METHODS

Animals and Management

Thirty-six (36) landrace pigs weighing on average 11 ± 1.02 kg were acquired and used in the study. The animals on arrival at the teaching and research farm of the Rivers State University were randomly allotted to their individual pens and pre-conditioned for one week following the method of Berepubo et al., (1994). All animals were similarly managed during the pre-conditioning period. Thus, during the period the animals were similarly fed and properly managed to properly adapt them to their new environment. Their environments were constantly kept cleaned throughout the study duration. Pigs were presented to four dietary treatments with 9 pigs per treatment for 4 weeks during the toxicological phase.

Crude oil contamination

The crude oil used in this study is the Bonny Light obtained from Agip Oil Company Nigeria Limited. Prior to using the crude oil in contaminating the experimental diets, it was treated according to the method of Berepubo et al., (1994) to enable its light fractions to evaporate leaving the stable product that mimics natural crude oil during pollution.

Experimental diets

All animals were fed a similar diet (Pfizer grower mash™) except that they contained different levels of dietary crude oil: T_1 (the control diet contained 0gram of crude/kg of diet, T_2 (contained 5gram of crude oil/kg of diet), T_3 (contained 10gram crude oil/kg of diet) and T_4 (contained 15gram/kg of diet), respectively. These diets were tagged the control, low, medium and high levels of crude oil-contaminations, respectively. Animals received their respective experimental diets for 4 weeks.

Blood sample collections

At the end of the 4 weeks, blood samples were collected from individual pig from each of the four dietary treatment groups into ethylene diamine tetracetic acid (EDTA) treated tubes between 9 and 10 am and immediately snap-frozen for later analyses by hematology auto-analyzer (BC-2300). Hb concentration, PCV, RBC count and total and differential WBC counts of each group were finally determined using standard laboratory procedures. This was the first phase here referred to the as the 'toxicological phase' and results confirmed changes in the hematological characteristics of the pigs in a linear fashion as the levels of dietary crude oil increased in the diets (results not shown here). The objectives of this study are to test the hypothesis of the efficacy of the protective effect of vitamin E alone or vitamin E in combination with selenium in ameliorating the toxicological effects of crude oil on the hematological characteristics of growing pigs fed increasing gradient crude oil-contaminated diets.

Thus, after collating the hematological data in the toxicological phase, the ameliorative phase involving vitamin E and selenium immediately commenced with the same group of animals; however, 3 pigs from the control group only were enrolled in this phase as the overall control whereas the 9 pigs in the positive control groups were randomly allotted in this phase into the various treatment groups in this ameliorative phase, respectively. Thus, in this phase, there were 10 treatments of 3 pigs each as: T_0, (5_0, $5_{vit\ E}$, $5_{vit\ E+Se}$), (10_0, $10_{vit\ E}$, $10_{vit\ E+Se}$) and (15_0, $15_{vit\ E}$, $15_{vit\ E+Se}$). That is, T_0 = overall control, 5_0, $5_{vit\ E}$, $5_{vit\ E+Se}$ treatment 2 of 5g of crude oil-contamination/kg of diet but in this ameliorative phase 5_0, diet contained no crude oil again in their diet, $5_{vit\ E}$, diet contained no crude oil but 200mg of vitamin E only and $5_{vit\ E+Se}$ diet contained no crude oil but 200mg of vitamin E + 5mg of Se/kg of diet,

respectively. These descriptions were the same for the 10 and 15 g of crude oil-contaminated diets for vitamin E and Se, respectively. As was with the 'toxicological phase', this phase also lasted for 4 weeks. Therefore, as was done in the toxicological phase, at the end of the 4 weeks in this phase blood samples from the 3 pigs in each of the treatment group were collected for Hb concentration, PCV, RBC count and total and differential WBC counts for final determination also using standard laboratory procedures.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS. Treatment means were compared using Tukey's test. The experimental design was the CRD. Therefore, the model was $Y_{ij} = \mu + X_i + E_{ij}$; where: Y_{ij} = individual observation of any animal receiving a treatment, μ = population mean, X_i = effect of the i^{th} diet (i = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) and E_{ij} = the error term. An *α-level* of *0.05* was used for all statistical comparisons to detect significance.

3. RESULTS

The results of the amelioration influence of vitamin E and selenium on Hb, RBC counts and PCV (Table 1). The blood parameters of growing pigs administered varied levels of crude oil-contaminated diets after four weeks of withdrawal and ameliorated with vitamin E alone or in combination with Se showed that the Hb concentrations of pigs remedied with vitamin E alone were higher ($P < 0.05$) than their control counterparts, particularly with the low (5g) and medium (10g) levels of dietary crude oil contamination diets. Nevertheless, for the high (15g) dietary crude oil intake Hb values were similar ($P > 0.05$) for vitamin E alone or vitamin E + selenium remedied animals with the overall control. Similar patterns were observed with the RBC counts and PCV concentrations, respectively. The results of the amelioration effect of vitamin E or vitamin E + Se on the WBC counts and their differentials (Table 2).

Table 1 Amelioration effects of vitamin E or vitamin E + Se on Hb, RBC and PCV parameters of growing pigs fed crude oil-contaminated diets

Treatments												
Item	T_0	5_0	$5_{vit\ E}$	$5_{vit\ E+Se}$	10_0	$10_{vit\ E}$	$10_{vit\ E+Se}$	15_0	$15_{vit\ E}$	$15_{vit\ E+Se}$	SEM	*P-value*
Hb (g/dl)	13.7^a	11.3^b	15.8^c	15.0^c	11.7^b	15.0^c	15.2^c	10.0^b	13.3^a	13.5^a	0.5	0.002
RBC (ul^3)	6.3^a	5.5^b	7.0^c	6.7^a	5.4^b	6.5^a	7.5^c	4.8^d	6.2^a	5.8^a	0.2	0.001
PCV (%)	41.3^a	34.7^b	42^a	45^a	35.3^b	42^a	43^a	30^c	42.3^a	45^a	1.5	0.001

$^{a, b, c, d}$ means within the same row with different superscripts are significantly ($P<0.05$) different

Table 2 Amelioration effects of vitamin E + Se on WBC counts and their differentials of growing pigs fed crude oil-contaminated diets

Treatments												
Item	T_0	5_0	$5_{vit\ E}$	$5_{vit\ E+Se}$	10_0	$10_{vit\ E}$	$10_{vit\ E+Se}$	15_0	$15_{vit\ E}$	$15_{vit\ E+Se}$	SEM	*P-value*
WBC (ul^3)	6.2^a	6.6^a	6.5^a	6.4^a	8.4^b	8.5^b	8.6^b	8.5^b	9.1^b	8.8^b	0.2	0.003
NEU (%)	46.7^a	36.7^b	42.7^c	66.7^d	38.0^b	59.3^c	52.3^c	48.0^a	45.0^a	55.0^c	1.0	0.002
LYM (%)	50.7^a	62.0^b	54.3^c	32.7^d	59.3^b	39.3^c	45.0^c	50.7^a	54.7^c	40.7^c	1.2	0.001
EOS (%)	0.6	1.0	1.3	0.6	2.0	1.0	1.3	1.0	0.3	1.0	0.5	0.514
MONO (%)	2.0^a	0.3^b	1.7^a	0.0^b	0.7^b	0.4^b	1.3^c	0.3^b	0.0^b	3.3^c	0.3	0.000

$^{a, b, c, d}$ means within the same row with different superscripts are significantly ($P < 0.05$) different

In the WBC counts, vitamin E alone was able again to fully ameliorate the toxicological effects of the ingested dietary crude oil as the WBC counts of the low dietary crude oil diet animals and those of the control were similar ($P > 0.05$). However, for the medium and high concentrations of dietary crude oil diets vitamin E alone significantly ($P < 0.05$) ameliorated or improved WBC counts as the counts of the WBC of the medium and high dietary crude oil contents were significantly ($P < 0.05$) higher compared with controls. This pattern was also mirrored by WBC differentials.

4. DISCUSSION

In this study, it was initially confirmed that the hematological characteristics of the growing pigs were toxicologically affected as evidenced by their reduced values by the ingested dietary crude oil, especially at the medium and high levels of dietary ingestion of

the crude oil. In other words, Hb, RBC, PCV and WBC of the positive control groups were significantly reduced signifying toxicities of the animals by the ingested crude oil. However, the use of vitamin E and selenium completely restored to normalcy the hematological characteristics at the various levels of crude oil addition/kg to the diet. This pattern is overwhelmingly true for RBC and PCV in particular.

Therefore, to this point as earlier stated, it was observed that vitamin E restored the blood properties to normalcy. However, when vitamin E in combination with selenium was used there were no further significant improvements in the blood properties. This observation therefore is not in tandem with literature data regarding the synergistic relationship between vitamin E and selenium in dealing with oxidative stress, such as the one induced by crude oil in the study in the improvement of the health status of the animal (Aslam et al., 2010). However, the finding in this study is in tandem with the data of Achuba and Awhin, (2008) and Manju and Sushovan, (2017) that found that vitamin E and C either separately or in combination restored RBC count, Hb concentration and WBC counts to normal values in goats and in rabbits after crude oil and arsenic intoxications, suggesting that vitamins alone, such as vitamins E and C alone or in combinations are very effective and efficient antioxidants in protecting animals.

Again, Ognjanovic et al., (2003) that investigated the effect of pretreatment with vitamin E demonstrated that vitamin E pretreatment significantly reduced the toxic effects of cadmium in rodents' hematological properties thereby further supports the efficacy of vitamin E in reversing toxicant negative effect in the blood characteristics of the animal. Additionally, Gao et al., (2010) in their studies showed that vitamin E supplementation alleviated the oxidative stress induced by dexamethasone in broiler chickens and in the process protected the animals against anaemia and illness. Therefore, our findings that vitamin E ameliorated the toxic effects of the ingested dietary crude oil was not surprising. It is therefore, not a gainsaying that vitamin E is an efficient antioxidant agent in managing oxidative stress in the animal.

5. CONCLUSIONS

Dietary crude oil was toxic on the hematological characteristics of the growing pig. Vitamin E alone ameliorated the toxicological effects of crude oil on the hematological parameters of the pig. This was because the degree of amelioration was not further enhanced when vitamin E was administered together with selenium, even at the 15 g (high intake) crude oil/kg of diet.

Informed consent

Not applicable.

Ethical approval

The Animal ethical guidelines are followed in the study for species observation & experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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